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# *Toxares koreanus* sp. nov. – a new *Toxares* species from South Korea (Hymenoptera, Braconidae, Aphidiinae)

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## Abstract

The genus *Toxares* Haliday, 1840 is a small taxon of Aphidiinae, consisting four valid species in the world. One *Toxares* species is recorded as new to science from South Korea, in this study. Descriptions and illustrations of the new species, *T. koreanus* **sp. nov.**, are provided, together with their mitochondrial *cytochrome c oxidase subunit I* (*COI*) and D2 region of the nuclear gene for *28S rRNA* (*28S*) sequences. The phylogenetic tree reconstructed using a combination of *COI* and *28S* revealed the phylogenetic position of the genus *Toxares* within Aphidiinae.

## Keywords

DNA barcoding, parasitoid wasps, phylogenetics, systematics, taxonomy

## Introduction

The genus *Toxares* Haliday, 1840 is a small genus of Aphidiinae with four known species from the Holarctic. *Toxares deltiger* (Haliday, 1833) was the first species to be described from the genus. For a long time, it was only known in Europe, but it has

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been recorded from the USA (Pike et al. 2000), Turkey (Tomanović et al. 2008) and several Asian countries (Korea, Kazakhstan, Pakistan, China, India) (Choi et al. 2017; Davidian 2018, 2020). Takada (1965) described *Toxares shigai* Takada, 1965 from Japan, and later Shuja-Udin (1974) described two additional species, *Toxares zakai* Shuja-Udin, 1974 and *Toxares macrosiphohagum* Shuja-Udin, 1974 from India. All known *Toxares* species were rarely sampled, and most records came from traps and net sweeping, and consequently without evidence about its aphid hosts. Nevertheless, a part of the records came from reared aphid colonies and available literature data, and it is confirmed that aphid hosts of *Toxares* species belong to the tribes Macrosiphini and Aphidini including some pest aphids (e.g. *Metopolophium dirhodum* (Walker) (Powell 1980, 1982; Dean et al. 1981; Starý et al. 1981; Höller et al. 1993), *Sitobion avenae* (Fabricius) (Dean et al. 1981; Powell 1982; Cameron et al. 1984), *Acyrthosiphon pisum* (Harris) (Cameron et al. 1984), *Myzus persicae* (Sulzer) (Mackauer 1968; Starý and Ghosh 1975, 1983; Marsh 1979; Hofsvang and Hågvar 1983), *Aphis craccivora* (Koch) (Raychaudfuri et al. 1990), *Schizaphis rotundiventris* (Signoret) (Starý and Ghosh 1983) and *Rhopalosiphum nymphaeae* (L.) (Starý and Ghosh 1983).

Based on the forewing venation being related to braconid ancestors, the genus *Toxares* is classified within the Ephedrini tribe (Mackauer 1961), which has sometimes been considered basal within Aphidiinae (Belshaw and Quicke 1997; Sanchis et al. 2000; Derocles et al. 2012). Some other studies showed that the tribe Praini is basal (Smith et al. 1999). However, there is very little evidence about molecular data of *Toxares* species, and its phylogenetic position is still unknown. Derocles et al. (2012) determined that the phylogenetic position of *Toxares deltiger* is between Ephedrini and Praini based on sequences of the *Cytochrome c Oxidase subunit I* (*COI*) gene. Ye et al. (2017) analysed molecular markers for identification of primary parasitoids of cereal aphids. Within their analysis, *T. deltiger* clustered as a sister group of the Trioxini tribe based on *COI* sequences and as a sister group of Aphidiini based on *16S* ribosomal RNA (Ye et al. 2017).

The aim of this study is to present additional knowledge about the diversity of *Toxares* species. After initial research of Korean aphid parasitoid fauna, we recognized a new *Toxares* species which is herein described and diagnostified using morphological and molecular characters. We also analysed phylogenetic relationships among genera *Toxares*, *Ephedrus* Haliday, 1833 and *Praon* Haliday, 1833 and discussed the phylogenetic position of the genus *Toxares* within Aphidiinae.

## Material and methods

### Specimen collection and morphological analysis

Specimens were collected by Malaise trap in a deciduous forest habitat (mostly *Quercus* spp.) in Mt. Beophwa which is about 450 m.a.s.l. *Rosa multiflora*, *Cirsium japonicum*, and *Urtica thunbergiana* were the dominant plant species. Two specimens were slide-mounted with Hoyer medium and one preserved in 70% ethanol. External structure

was studied and measurements taken with a LEICA DM LS phase-contrast microscope. Morphological terminology used in this paper regarding diagnostic characters is based on that of Sharkey and Wharton (1997).

## Molecular analysis

DNA extraction was performed using a LaboPass Tissue Kit (COSMOgenetech, Korea) following the manufacturer's protocol. In order to conserve morphologically complete voucher specimens, the DNA extraction method was slightly modified from the 'non-destructive method' by Favret (2005) and 'freezing method' by Yaakop et al. (2009). In the original protocol, the sample was crushed and then soaked in 180 µl of TL buffer + 20 µl of proteinase-K, followed by three hours of incubation at 55 °C. In the slightly modified DNA extraction methods, samples with all specimens were soaked in 180 µl of TL buffer + 20 µl of proteinase-K without destroying the sample, followed by 10 minutes incubation at 55 °C and kept in a freezer at -22 °C overnight. After that the general protocol was used for the remaining steps. The target site for molecular identification was the front partial region of mitochondrial *COI*, amplified using the primers LCO1490 (forward) 5'-GGTCAACAAATCATAAAGATAT-TGG-3' and HCO2198 (reverse) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). The molecular marker used for comparing with other *Toxares* species and species of *Ephedrus* and *Praon* was the D2 region of the nuclear gene for *28S rRNA* (*28S*rDNA), amplified using primers 28SD2f (forward) 5'-AGAGAGAGTTCAAGAGTA-CGTG-3' (Belshaw and Quicke 1997) and 28SD2r (reverse) 5'-TTGGTCCGTGTTTCAAGACG-GG-3' (Campbell et al. 1993). We used heterogenous F/R primers as referred to by Tomanović et al. (2018).

Polymerase chain reaction (PCR) amplification of *COI* and *28S* was conducted by using AccuPower PCR PreMix (Bioneer Corp., Daejeon, Korea) in 20 µl of a reaction mixture consisting of 3 µl of DNA extract, 2 µl of primer, and 15 µl of $H_2O$. Thermal profile for *COI* was as follows: denaturation for 5 min at 95 °C; 38 cycles of 20 s at 95 °C, 30 s at 45 °C, and 40 s at 72 °C; and final extension at 72 °C for 5 min. Thermal profile for *28S* was as follows: denaturation for 3 min at 95 °C; 32 cycles of 30 s at 95 °C, 30 s at 48 °C, and 30 s at 72 °C; and final extension at 72 °C for 10 min. The PCR products were tested by electrophoresis on agar gel and if a band existed, we commissioned Bionocs (Korea) for sequencing and purification.

Sequences were edited with FinchTV ver. 1.4.0 (www.geospiza.com), aligned with CLUSTAL W integrated in MEGA X (Kumar et al. 2018), and trimmed to lengths of 642 bp (*COI*), and 476 bp (*28S*). Sequences are deposited in GenBank under accession numbers: ON007269–ON007271 (*COI*), ON003419–ON003421 (28S). Additional sequences from GenBank (Fig. 3) were used for phylogenetic analysis.

Average genetic distances were calculated using MEGA X and Kimura's two-parameter method of base substitution (K2P, Kimura 1980) (Table 1).

MEGA X was used to construct phylogenetic trees based on each gene used in the study, as well as a combined tree employing concatenated sequences of both genes.

Phylogenetic relationships were reconstructed using Maximum Likelihood (ML) and Maximum Parsimony (MP) methods.

**Table 1.** Genetic distances (K2P) between analysed Aphidiinae species based on *COI* (bold) and *28S* (upper right) and on both genes combined (lower left).

| | *T. koreanus* (JS1) | *T. koreanus* (JS1-1) | *T. koreanus* (JS1-2) | *T. deltiger* | *E. helleni* | *E. nacheri* | *E. persicae* | *E. plagiator* | *P. abjectum* | *P. bicolor* | *P. dorsale* | *P. yomenae* | *V. canescens* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. koreanus* (JS1) | | **0.00** | **0.00** | **0.14** | **0.21** | **0.21** | **0.22** | **0.21** | **0.16** | **0.16** | **0.17** | **0.19** | **0.29** |
| | | 0.00 | 0.00 | 0.05 | 0.20 | 0.22 | 0.20 | 0.23 | 0.19 | 0.19 | 0.19 | 0.19 | 0.38 |
| *T. koreanus* (JS1-1) | 0.00 | | **0.00** | **0.14** | **0.21** | **0.21** | **0.22** | **0.21** | **0.16** | **0.16** | **0.17** | **0.19** | **0.29** |
| | | | 0.00 | 0.05 | 0.20 | 0.22 | 0.20 | 0.23 | 0.19 | 0.19 | 0.19 | 0.19 | 0.38 |
| *T. koreanus* (JS1-2) | 0.00 | 0.00 | | **0.14** | **0.21** | **0.21** | **0.22** | **0.21** | **0.16** | **0.16** | **0.17** | **0.19** | **0.29** |
| | | | | 0.05 | 0.20 | 0.22 | 0.20 | 0.23 | 0.19 | 0.19 | 0.19 | 0.19 | 0.38 |
| *T. deltiger* | 0.09 | 0.10 | 0.10 | | **0.23** | **0.22** | **0.20** | **0.23** | **0.18** | **0.19** | **0.18** | **0.20** | **0.31** |
| | | | | | 0.18 | 0.21 | 0.18 | 0.21 | 0.19 | 0.18 | 0.18 | 0.18 | 0.38 |
| *E. helleni* | 0.21 | 0.21 | 0.21 | 0.21 | | **0.10** | **0.20** | **0.09** | **0.22** | **0.21** | **0.22** | **0.23** | **0.29** |
| | | | | | | 0.06 | 0.05 | 0.06 | 0.22 | 0.22 | 0.22 | 0.23 | 0.39 |
| *E. nacheri* | 0.21 | 0.21 | 0.21 | 0.21 | 0.08 | | **0.22** | **0.03** | **0.23** | **0.21** | **0.21** | **0.21** | **0.27** |
| | | | | | | | 0.08 | 0.00 | 0.21 | 0.22 | 0.22 | 0.21 | 0.41 |
| *E. persicae* | 0.21 | 0.21 | 0.21 | 0.20 | 0.13 | 0.16 | | **0.22** | **0.22** | **0.21** | **0.22** | **0.22** | **0.32** |
| | | | | | | | | 0.08 | 0.20 | 0.20 | 0.20 | 0.21 | 0.36 |
| *E. plagiator* | 0.21 | 0.21 | 0.21 | 0.22 | 0.08 | 0.02 | 0.16 | | **0.24** | **0.21** | **0.22** | **0.21** | **0.27** |
| | | | | | | | | | 0.22 | 0.22 | 0.22 | 0.22 | 0.42 |
| *P. abjectum* | 0.17 | 0.17 | 0.17 | 0.17 | 0.22 | 0.23 | 0.21 | 0.23 | | **0.07** | **0.07** | **0.10** | **0.32** |
| | | | | | | | | | | 0.00 | 0.00 | 0.02 | 0.42 |
| *P. bicolor* | 0.17 | 0.17 | 0.17 | 0.18 | 0.22 | 0.22 | 0.21 | 0.22 | 0.04 | | **0.03** | **0.08** | **0.29** |
| | | | | | | | | | | | 0.00 | 0.02 | 0.42 |
| *P. dorsale* | 0.17 | 0.17 | 0.17 | 0.18 | 0.22 | 0.22 | 0.21 | 0.22 | 0.04 | 0.02 | | **0.08** | **0.28** |
| | | | | | | | | | | | | 0.02 | 0.42 |
| *P. yomenae* | 0.18 | 0.18 | 0.18 | 0.19 | 0.22 | 0.22 | 0.22 | 0.22 | 0.06 | 0.05 | 0.05 | | **0.28** |
| | | | | | | | | | | | | | 0.44 |
| *V. canescens* | 0.32 | 0.32 | 0.32 | 0.33 | 0.32 | 0.32 | 0.33 | 0.32 | 0.361 | 0.34 | 0.34 | 0.34 | |

## Results

### Description of the new species

***Toxares koreanus* Tomanović, Kim & Petrović, sp. nov.**
https://zoobank.org/82B185A3-71D2-4E6B-B84B-AC887FC14140

**Diagnosis.** *Toxares koreanus* sp. nov. morphologically resembles *T. shigai* in having elongated flagellomere 1 ($F_1$), which is clearly longer than flagellomere 2 ($F_2$) and elongated petiole at the spiracles level. However, *T. koreanus* sp. nov. is easily distinguished from *T. shigai* in the shape of petiole (petiole with parallel sides in *T. koreanus* sp. nov., while laterally expanded and longitudinally striated in *T. shigai*), yellow colored $F_1$–$F_3$ and even a yellowish base of $F_4$ in *T. koreanus* sp. nov., while light brown colored $F_1$–$F_3$ in *T. shigai*. Also, *T. koreanus* sp. nov. morphologically resembles *T. macrosiphophagum*, but differs in more elongated $F_1$ which is clearly longer than $F_2$, more elongated

petiole, and color of basal flagellomeres (yellow colored $F_1$–$F_3$ and yellow base of $F_4$ in *T. koreanus* sp. nov., and yellowish $F_1$ and $F_2$ in *T. macrosiphophagum*).

**Description. Female** (Fig. 1, Suppl. material 1: Fig. S1): ***Head*** (Fig. 1A) rounded, bearing sparse setae. Eyes large and oval. Tentorial index (tentoriocular line/intertentorial line) 0.30–0.36. Clypeus with about 10 long setae. Malar space equal to 0.20 of longitudinal eye diameter. Mandible bidentate, with 6–7 setae on the outer surface. Maxillary palps with four palpomeres, labial palps with three palpomeres. Antenna 17–18 segmented (17, 2♀; 18, 1♀), flagellate (Fig. 1B). Flagellomere 1 ($F_1$) (Fig. 1C) clearly longer than $F_2$ ($F_1/F_2$ length 1.1–1.2) and 3.75–4.00 times as long as its maximum width at the middle. $F_1$ with 2–3 and $F_2$ with 3–5 longitudinal placodes (Fig. 1C). Flagellomeres covered uniformly with semi-erect setae subequal to antennal segments diameter.

***Mesosoma.*** Mesoscutum smooth, rounded, with mid pit in the middle posterior part. Notaulices distinct in very short ascendent portion of anterolateral margin, with two rows of long setae along the dorsolateral part of mesoscutum (Fig. 1D). Scutellum elongated, bearing 6–7 long setae in the central part. Scutellar sulcus divided into equal halves. Propodeum (Fig. 1E) areolated with large central areola. Upper propodeal areolas with 5–7 long setae and lower areolas with 1–4 long setae on each. Forewing (Fig. 1F) densely pubescent, with long marginal setae. Pterostigma elongated, 6.35–6.7 times as long as its width, subequal to R1 vein (Fig. 1F). Forewing 2RS vein shorter than 3RSa vein (2RS/3RSa = 0.55) and 3RSa shorter than 3RSb vein (3RSa/3RSb = 0.73).

***Metasoma.*** Petiole (Fig. 1G) slightly rugose and convex dorsally, with lateral depression at level of prominent spiracular tubercles. Petiole length 2.80–2.86 times its width at the base of spiracles, with 5–6 long setae along each side (Fig. 1G). Ovipositor sheath deltoid shaped (Fig. 1H).

***Body length*:** about 1.70–2.20 mm.

***Coloration.*** General body color light brown to yellow. Scape, pedicel and $F_1$–$F_3$ yellow, $F_4$ basally light brown, remaining antennal parts brown. Mouthparts yellow. Head brown. Mesoscutum light brown to brown. Propodeum light brown. Legs yellow with brown apices. Petiole yellow to light brown, other metasomal terga light brown. Ovipositor sheath yellow.

**Male** (Fig. 2): Antenna 19-segmented with shorter flagellomeres (Fig. 2A). $F_1$ about 2.60 times as long as wide and longer than $F_2$ (Fig. 2B). Number of longitudinal placodes on $F_1$ and $F_2$, 3 and 5, respectively. Maxillary palps with four palpomeres, labial palps with three palpomeres. Pterostigma shorter than in female and about 4.7 times as long as wide. Mesosoma with small mid pit . Petiole shorter than in female and about 2.55 times longer than width at spiracles level. Male genitalia (Fig. 2C). Body generaly darker than in female. Scapus and pedicel light brown. $F_1$ yellow, remaining antennal parts brown. Legs yellow to light brown with dark apices. Petiole and first half of metasomal terga light brown, remaining part of metasoma brown. Legs and mouthparts light brown.

**Etymology.** The name of the new species is derived from Republic of Korea where it was found.

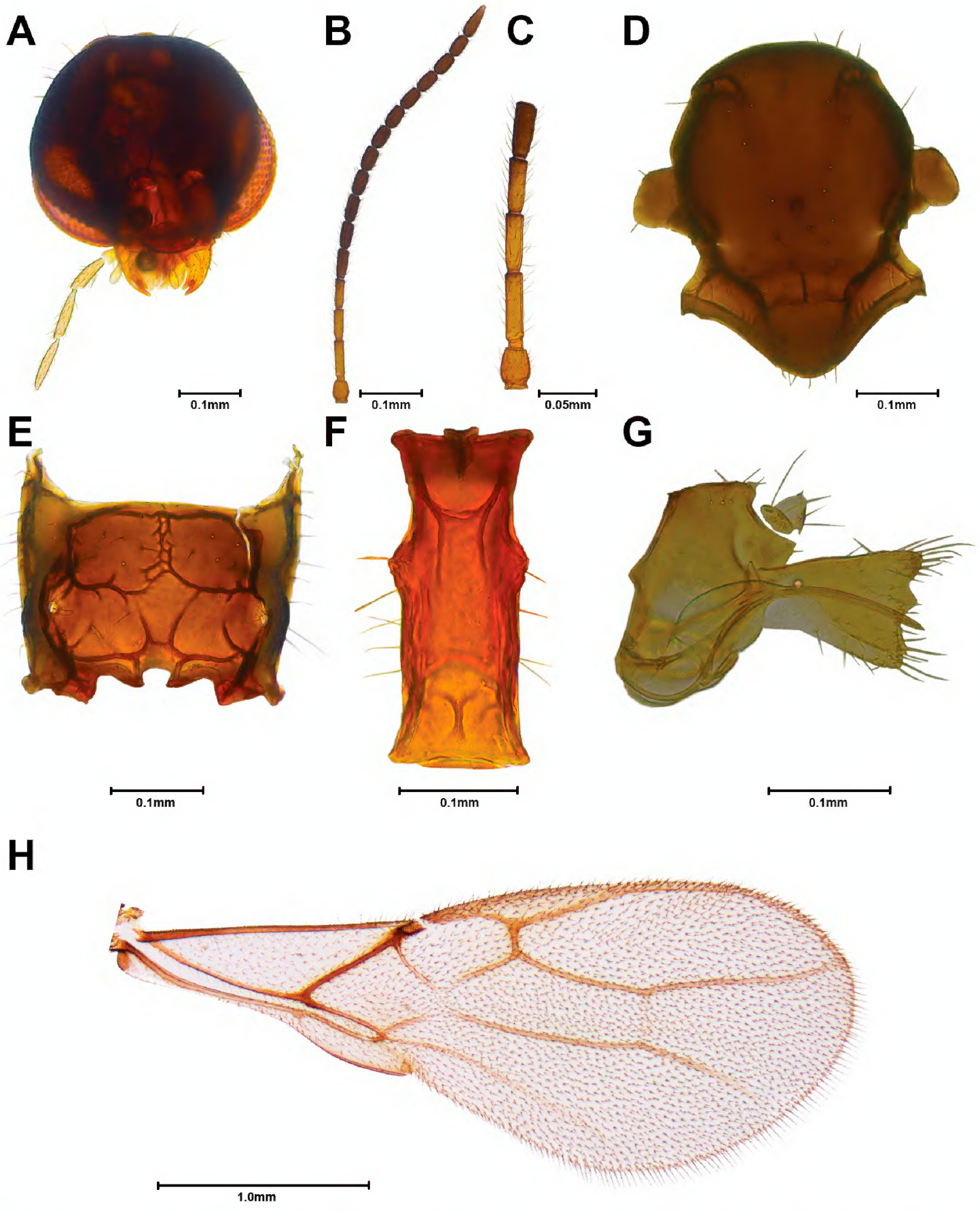


**Figure 1.** *Toxares koreanus* sp. nov., female **A** head **B** antennae **C** flagellomere $F_1$–$F_4$ **D** mesoscutum **E** propodeum **F** petiole **G** ovipositor **H** forewing.

**Specimens examined.** ***Holotype*:** Korea • 1 ♀; Mt. Beophwa, San 128-1, Wolgok-ri, Cheoncheon-myeon, Jasnsu-gun, Jeollabuk-do; 35°42'07.6"N, 127°31'54.7"E; collected by Malaise trap: 06.V–24.V.2021; leg. Yeonghyeok Yu, Sangjin Kim, JuHyeong Sohn, Yunjong Han, Gyeongyeon Lee. Holotype deposited in National Institute of Biological Resources, Incheon, Republic of Korea slide mounted.

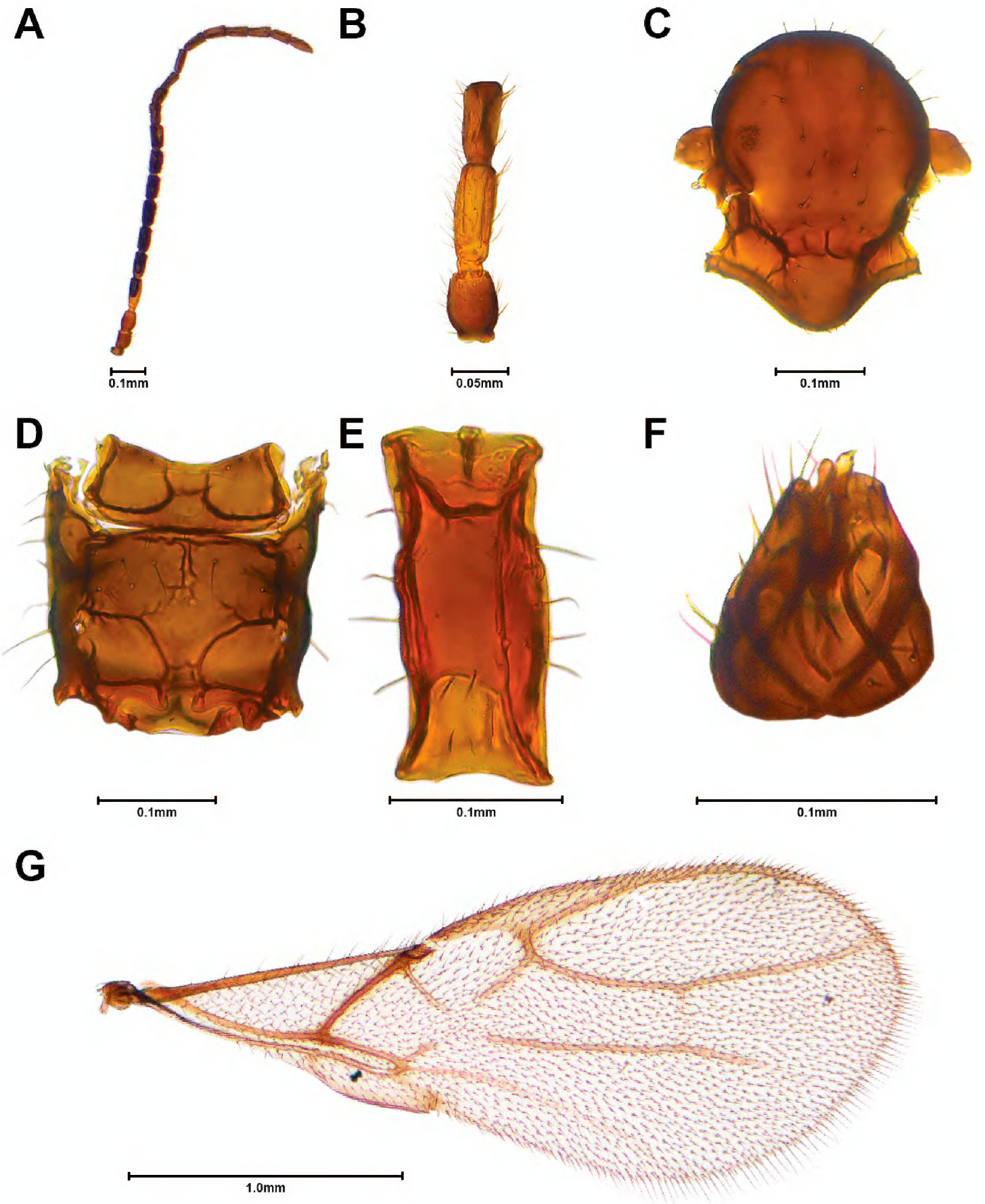


**Figure 2.** *Toxares koreanus* sp. nov., male **A** antennae **B** flagellomere $F_1$ and $F_2$ **C** mesoscutum **D** propodeum **E** petiole **F** ovipositor **G** forewing.

***Paratypes*:** Korea • 1 ♂; Mt. Beophwa, San 128-1, Wolgok-ri, Cheoncheon-myeon, Jasnsu-gun, Jeollabuk-do; 35°42'07.6"N, 127°31'54.7"E; collected by Malaise trap: 06.V–24.V.2021; leg. Yeonghyeok Yu, Sangjin Kim, JuHyeong Sohn, Yunjong Han, Gyeongyeon Lee. Paratype slide mounted and deposited in National Institute of Biological Resources, Incheon, Republic of Korea.

**Additional material.** Korea • 2 ♀; 1 ♀, Mt. Beophwa, San 128-1, Wolgok-ri, Cheoncheon-myeon, Jasnsu-gun, Jeollabuk-do; 35°42'07.6"N, 127°31'54.7"E; collected by Malaise trap: 06.V–24.V.2021; leg. Yeonghyeok Yu, Sangjin Kim, JuHyeong Sohn, Yunjong Han, Gyeongyeon Lee • 1 ♀, same locality; collected by Malaise trap: 24.V–02.VI.2021; leg. Yeonghyeok Yu, Sangjin Kim, JuHyeong Sohn, Yunjong Han, Gyeongyeon Lee. Specimens deposited dry and immersion-mounted in Kunsan National University, Jeollabuk-do, Republic of Korea.

**Molecular analysis.** Obtained phylogenetic trees reconstructed based on *COI*, *28S* and the combination of both genes showed identical topology, and the tree based on the combination of both genes is shown on Fig. 3. *Toxares koreanus* sp. nov. groups with the only other *Toxares* species used in the analysis, while this clade is sister to the clade of *Praon* species. *Ephedrus* species basally form a separate clade on the tree.

Calculated genetic distances (Tables 1, 2) also indicate closer relatedness between *Toxares* and *Praon* than between *Toxares* and *Ephedrus*.

## Discussion

The genus *Toxares* is considered as one of the most basal within the subfamily Aphidiinae, classified within the tribe Ephedrini (Mackauer 1961), and sharing a braconid ancestral wing venation pattern with species of the genus *Ephedrus*. Except for the forewing venation pattern as a clear plesiomorphy, the newly described species, along with other congeners (e.g. *T. deltiger*), shares additional plesiomorphic character states, such as a large number of placodes on $F_1$ and $F_2$, areolated propodeum, and 4-maxillary and 3-labial palpomeres. On the other hand, the elongated flagellomeres and petiole represent apomorphic characters (Tomanović et al. 2006). *Toxares koreanus* sp. nov. also possesses a small mid pit on the mesoscutum. This is a unique character present only in some *Ephedrus* species from the subgenus *Fovephedrus* (Chen 1986; Kocić et al. 2019), as well as in all known *Toxares* species. *Toxares koreanus* sp. nov. along with other congeners (e.g. *T. deltiger*) possesses a divided scutellar sulcus (Fig. 1D), a character state present in the subgenus *Breviephedrus* (e.g. *E. brevis*) (Kocić et al. 2019), which supports the phylogenetic position of the genus *Toxares* within the tribe Ephedrini.

**Table 2.** Genetic distances (K2P) between genera *Toxares*, *Ephedrus* and *Praon*.

| | **Within group mean distances** | | |
|---|---|---|---|
| | ***COI*** | ***28S*** | **combined** |
| *Toxares* | 0.07 | 0.02 | 0.05 |
| *Ephedrus* | 0.14 | 0.06 | 0.10 |
| *Praon* | 0.07 | 0.01 | 0.04 |
| | **Between group mean distances (COI/ 28S/ combined)** | | |
| | ***Toxares*** | ***Ephedrus*** | ***Praon*** |
| *Toxares* | | | |
| *Ephedrus* | 0.21/ 0.21/ 0.21 | | |
| *Praon* | 0.19/ 0.18/ 0.17 | 0.22/ 0.22/ 0.22 | |

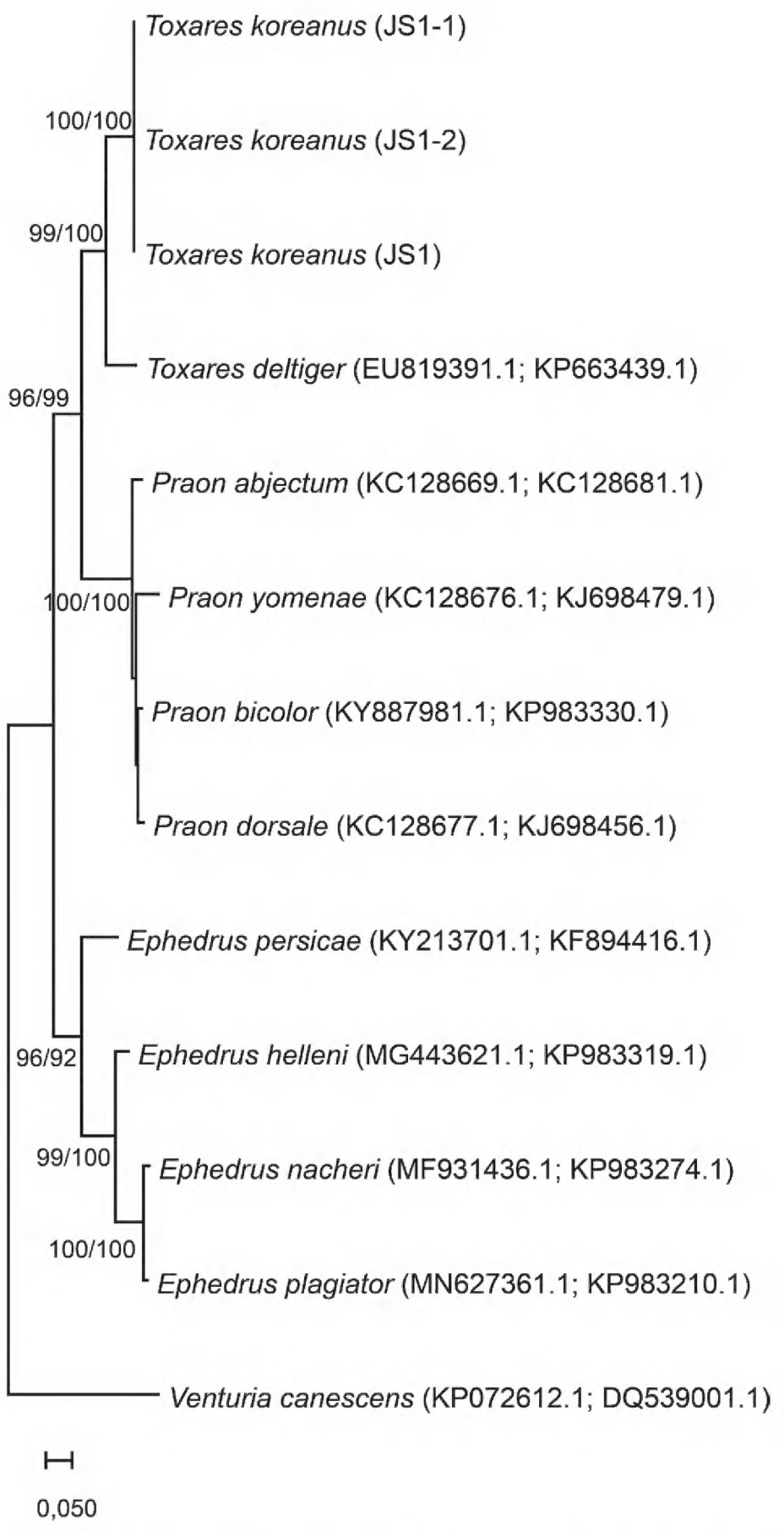


**Figure 3.** Phylogenetic relationships between *Toxares*, *Ephedrus* and *Praon* species based on combined sequences of *COI* and *28S RNA* genes. Species name is followed by code or GenBank accession numbers in brackets. Bootstrap values are indicated above/below branches in order ML/MP.

*Toxares koreanus* sp. nov. is the fifth known member of the genus *Toxares* and fourth species described from Asia. Based on the currently available data about the distribution of described species, we can assume that the origin of this genus should be Far Eastern Asia. Considering the habitat and plant diversity in Far Eastern Asia, we can expect to discover additional species of the genus *Toxares*.

Molecular analysis using *COI* and *28S* supports the description of the new species. *Toxares koreanus* sp. nov. is clearly separated from *T. deltiger* by both genes (Fig. 3, Table 1), in addition to morphological differences.

Molecular markers employed in this study show some incongruence with morphological characters. While *Toxares* is morphologically most similar to *Ephedrus*, molecular data suggests the genus is closer to *Praon* (Fig. 3, Tables 1, 2). Calculated genetic distances between all three genera are very high, based on both genes used in the analysis (Table 2). Although those between *Toxares* and *Ephedrus* are slightly higher than those between *Toxares* and *Praon*, it is still advisable to interpret these results carefully, and use an integrative approach including biological and ecological traits when making conclusions about the relatedness of groups. The discrepancy between morphological and molecular data is a fairly common occurence in Aphidiinae research and numerous studies have shown that molecular and morphological analyses often give somewhat conflicting results (Tomanović et al. 2013, 2018; Petrović et al. 2015; Jamhour et al. 2016; Čkrkić et al. 2020). One possible solution to this ongoing dilemma could be the use of more molecular markers or increasing the number of molecular operational taxonomic units, in an effort to uncover the mechanisms underlying the differences in multi-locus determined morphological traits (Zimmerman et al. 2000; Mezey et al. 2005; Čkrkić et al. 2020) and more emphasis on functions and adaptiation of morphological characters.

Although the genus *Toxares*, as a member of Ephedrini tribe, is already considered as basal within Aphidiinae (Mackauer 1961), our molecular data do not confirm it. We believe that discoveries of more species of this poorly known genus, along with appropriate molecular studies (which will include "ancient" genera *Pseudephedrus* and *Choreopraon*) should allow us to determine the exact phylogenetic position of *Toxares*.

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## References

Belshaw R, Quicke DL (1997) A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). Molecular Phylogenetics and Evolution 7(3): 281–293. https://doi.org/10.1006/mpev.1996.0400

Cameron PJ, Powell W, Loxdale HD (1984) Reservoirs for *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae), a polyphagous parasitoid of cereal aphids (Hemiptera: Aphididae). Bulletin of entomological research 74(4): 647–656. https://doi.org/10.1017/S0007485300014024

Campbell BC, Steffen-Campbell JD, Werren JH (1993) Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. Insect Molecular Biology 2(4): 225–237. https://doi.org/10.1111/j.1365-2583.1994.tb00142.x

Chen JH (1986) *Fovephedrus*, a new genus with four new species (Hymenoptera, Aphidiidae). Journal of Fujian Agricultural College 15: 91–100.

Choi S, Ku DS, Kim H (2017) New record of the genus *Toxares* Haliday (Hymenoptera: Braconidae: Aphidiinae) from Korea. Journal of Asia-Pacific Biodiversity 10(4): 596–598. https://doi.org/10.1016/j.japb.2017.07.004

Čkrkić J, Petrović A, Kocić K, Mitrović M, Kavallieratos NG, van Acthterberg C, Hebert PDN, Tomanović Ž (2020) Phylogeny of the subtribe Monoctonina (Hymenoptera, Braconidae, Aphidiinae). Insects 11(3): e160. https://doi.org/10.3390/insects11030160

Davidian EM (2018) Check-list of the aphidiid-wasp subfamily Ephedrinae (Hymenoptera, Aphidiidae) from Russia and adjacent countries. Entomological Review 98: 1091–1104. https://doi.org/10.1134/S0013873818080158

Davidian EM (2020) New data on the distribution of rare and little known Aphidiid wasps (Hymenoptera, Aphidiidae). Entomological Review 100: 1102–1105. https://doi.org/10.1134/S0013873820080072

Dean GJ, Jones MG, Powell W (1981) The relative abundance of the hymenopterous parasites attacking *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (F.) (Hemiptera: Aphididae) on cereals during 1973–79 in southern England. Bulletin of Entomological Research 71(2): 307–315. https://doi.org/10.1017/S0007485300008336

Derocles SAP, Le Ralec A, Plantegenest M, Chaubet B, Cruaud C, Cruaud A, Rasplus JY (2012) Identification of molecular markers for DNA barcoding in the Aphidiinae (Hym. Braconidae). Molecular Ecology Resources 12(2): 197–208. https://doi.org/10.1111/j.1755-0998.2011.03083.x

Favret C (2005) New non-destructive DNA extraction and specimen clearing technique for aphids (Hemiptera). Proceedings of the Entomological Society of Washington 107(2): 469–470.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Hofsvang T, Hågvar EB (1983) Primary parasitoids (Hym., Aphidiidae) and hyperparasitoids on aphids from Norway. Fauna Norvegica = Norwegian Journal of Entomology series B30: 60–62.

Höller C, Borgemeister C, Haardt H, Powell W (1993) The relationship between primary parasitoids and hyperparasitoids of cereal aphids: an analysis of field data. Journal of Animal Ecology 62(1): 12–21. https://doi.org/10.2307/5478

Jamhour A, Mitrović M, Petrović A, Starý P, Tomanović Ž (2016) Re-visiting the *Aphidius urticae* s. str. group: Re-description of *Aphidius rubi* Starý and *A. silvaticus* Starý (Hymenoptera:

Braconidae: Aphidiinae). Zootaxa 4178(2): 278–288. https://doi.org/10.11646/zootaxa.4178.2.6

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581

Kocić K, Petrović A, Čkrkić J, Mitrović M, Tomanović Ž (2019) Phylogenetic relationships and subgeneric classification of European *Ephedrus* species (Hymenoptera, Braconidae, Aphidiinae). ZooKeys 878: 1–22. https://doi.org/10.3897/zookeys.878.38408

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Mackauer M (1961) Die Gattungen der Familie Aphidiidae und ihre verwandtschaftliche Zuordnung (Hymenoptera: Ichneumonoidea). Beiträge zur Entomologie = Contributions to Entomology 11: 792–803. https://doi.org/10.21248/contrib.entomol.11.7-8.792-803

Mackauer M (1968) Insect parasites of the green peach aphid, *Myzus persicae* Sulz., and their control potential. Entomophaga 13(2): 91–106. https://doi.org/10.1007/BF02371780

Marsh PM (1979) Braconidae. Aphidiidae. Hybrizontidae. In: Krombein KV, Hurd JPD, Smith DR, Burks BD (Eds) Catalog of hymenoptera in America north of Mexico. Smithsonian Institution Press (Washington) 1: 144–313. https://doi.org/10.5962/bhl.title.5074

Mezey JG, Houle D, Nuzhdin S (2005) Naturally segregating Quantitative Trait Loci affecting wing shape of *Drosophila melanogaster*. Genetics 169(4): 2101–2113. https://doi.org/10.1534/genetics.104.036988

Petrović A, Mitrović M, Ivanović A, Ţikić V, Kavallieratos NG, Starý P, Bogdanović AM, Tomanović Ž, Vorburger C (2015) Genetic and morphological variation in sexual and asexual parasitoids of the genus *Lysiphlebus* - An apparent link between wing shape and reproductive mode. BMC Evolutionary Biology 15(5): 1–12. https://doi.org/10.1186/s12862-015-0293-5

Pike KS, Starý P, Miller T, Graf G, Allison D, Boydston L, Miller R (2000) Aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Northwest USA. Proceedings of the Entomological Society of Washington 102(3): 688–740.

Powell W (1980) *Toxares deltiger* (Haliday) (Hymenoptera: Aphidiidae) parasiting the cereal aphid, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) in southern England: a new host-parasitoid record. Bulletin of Entomological Research 70(3): 407–409. https://doi.org/10.1017/S0007485300007665

Powell W (1982) The identification of hymenopterous parasitoids attacking cereal aphids in Britain. Systematic Entomology 7(4): 465–473. https://doi.org/10.1111/j.1365-3113.1982.tb00457.x

Raychaudhuri D, Samanta AK, Pramanik DR, Tamili DK, Sarkar S (1990) Aphidiids (Hymenoptera) of Northeast India. Indira Publishing House (Michigan): 1–155.

Sanchis A, Latorre A, González-Candelas F, Michelena JM (2000) An 18S rDNA-based molecular phylogeny of Aphidiinae (Hymenoptera: Braconidae). Molecular Phylogenetics and Evolution 14(2): 180–194. https://doi.org/10.1006/mpev.1999.0701

Sharkey MJ, Wharton RA (1997) Morphology and terminology In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Manual of the New World Genera of the Family Braconidae. International Society of Hymenopterists Special Publication I (Washington DC), 433.

Shuja U (1974) Two new species of the genus *Toxares* Westwood (Aphidiidae: Hymenoptera) from India with a note on the genus. Indian Journal of Entomology 36: 268–274.

Smith PT, Kambhampati S, Volkl W, Mackauer M (1999) A phylogeny of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) inferred from mitochondrial NADH1 dehydrogenase gene sequence. Molecular Phylogenetics and Evolution 11(2): 236–245. https://doi.org/10.1006/mpev.1998.0575

Starý P (1981) Biosystematic synopsis of parasitoids on cereal aphids in the western Palaearctic (Hymenoptera, Aphidiidae; Homoptera, Aphidoidea). Acta entomologica bohemoslovaca 78(6): 382–396.

Starý P, Ghosh AK (1975) Aphid parasites (hymenoptera: Aphidiidae) from meghalaya, India. Oriental Insects 9(3): 343–349. https://doi.org/10.1080/00305316.1975.10434504

Starý P, Ghosh AK (1978) Further records of aphid parasitoids (Hymenoptera : Aphidiidae) from Meghalaya, India. Oriental Insects 12(1): 77–80. https://doi.org/10.1080/00305316.1978.10434553

Starý P, Ghosh AK (1979) Redescription of *Trioxys nepalensis* (Takada) and new host records of some aphid parasitoids (Hymenoptera : Aphidiidae) from Meghalaya, India. Oriental Insects 13(1–2): 41–45. https://doi.org/10.1080/00305316.1979.10433540

Starý P, Ghosh AK (1983) Aphid parasitoids of India and adjacent countries (Hymenoptera: Aphidiidae). Zoological Survey of India 7: 38–39.

Takada H (1965) A new species of the genus *Toxares* Haliday from Japan (Hymenoptera: Aphidiidae). Insecta matsumurana 28(1): 17–18.

Tomanović Ž, Kavallieratos NG, Starý P, Stanisavljević LŽ, Petrović-Obradović O, Tomanović S, Milutinović M (2006) Phylogenetic relationships among Praini (Hymenoptera : Braconidae : Aphidiinae) aphid parasitoids, with redescription of two species. Insect Systematics & Evolution 37: 213–226. https://doi.org/10.1163/187631206788831128

Tomanović Ž, Beyarslan A, Çetin Erdogan Ö, Žikić V (2008) New records of aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) from Turkey. Periodicum Biologorum 110(4): 335–338.

Tomanović Ž, Kos K, Petrović A, Starý P, Kavallieratos NG, Ţikić V, Jakše J, Trdan S, Ivanović A (2013) The relationship between molecular variation and variation in the wing shape of three aphid parasitoid species: *Aphidius uzbekistanicus* Luzhetzki, *Aphidius rhopalosiphi* De Stefani Perez and *Aphidius avenaphis* (Fitch) (Hymenoptera: Braconidae: Aphidiinae). Zoologischer Anzeiger 252(1): 41–47. https://doi.org/10.1016/j.jcz.2012.03.003

Tomanović Ž, Mitrović M, Petrović A, Kavallieratos NG, Ţikić V, Ivanović A, Rakhshani E, Starý P, Vorburger C (2018) Revision of the European *Lysiphlebus* species (Hymenoptera: Braconidae: Aphidiinae) on the basis of COI and 28SD2 molecular markers and morphology. Arthropod Systematics and Phylogeny 76(2): 179–213.

Yaakop S, van Achterberg C, Idris AB (2009) *Heratemis* Walker (Hymenoptera: Braconidae: Alysiinae: Alysiini): revision and reconstruction of the phylogeny combining molecular data and morphology. Tijdschrift voor Entomologie 152: 1–64. https://doi.org/10.1163/22119434-900000268

Ye Z, Vollhardt IM, Tomanovic Ž, Traugott M (2017) Evaluation of three molecular markers for identification of European primary parasitoids of cereal aphids and their hyperparasitoids. PLoS ONE 12(5): e0177376. https://doi.org/10.1371/journal.pone.0177376

Zimmermann E, Palsson A, Gibson G (2000) Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. Genetics 155(2): 671–683. https://doi.org/10.1093/genetics/155.2.671

## Supplementary material I

**Figure S1**

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Data type: Image (tif file)

Explanation note: Figure S1. Habitus of *Toxares koreanus* sp. nov., female.

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